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(54) Title: CHARGE REDUCTION IN ELECTROSPRAY MASS SPECTROMETRY

(57) Abstract

The charge state of ions produced by electrospray ionization is reduced in a controlled manner to yield predominantly singly charged ions through reactions with bipolar ions generated using a ^{210}Po alpha particle source or equivalent. The multiply charged ions generated by the electrospray undergo charge reduction in a neutralization chamber. The charge–reduced ions are then detected using a commercial orthogonal electrospray TOF mass spectrometer, although the neutralization chamber can be adapted to virtually any mass analyzer. The results obtained exhibit a signal intensity drop–off with increased oligonucleotides size similar to that observed with MALDI mass spectrometry, yet with the softness of ESI and without the off–line sample purification and pre–separation required by MALDI.

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Charge Reduction in Electrospray Mass Spectrometry

CROSS-REFERENCE TO RELATED APPLICATIONS

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MICROFICHE APPENDIX

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BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to electrospray ionization mass spectrometry, and more particularly to a method of charge reduction whereby ions produced by electrospray are amenable to neutralization and subsequent detection by time-of-flight mass spectrometry to yield high resolution mixture spectra.

2. Description of Related Art

The structure of deoxyribonucleic acid (DNA) consists of two parallel strands connected by hydrogen bonding. Double stranded DNA molecules assume a double helix structure with varying geometric characteristics. Under certain salt or temperature conditions, denaturation can occur and the two DNA strands become separated.

The order of nucleotides along a single strand corresponds to the sequence of DNA. Each set of three contiguous bases (a codon) encodes a particular amino acid used in protein synthesis. Successive codons are

organized into a gene to encode a particular protein. DNA is thus present in living cells as the fundamental genetic information carrier.

The human genome is the complete set of human DNA present in every cell (apart from reproductive and red blood cells). It is believed that total human DNA comprises 3 billion base pairs encoding about 100,000 genes. Sequencing the entire genome is desirable because knowledge of gene sequencing should increase the understanding of gene regulation and function and allow precise diagnostics and treatment of genetic diseases.

Using current sequencing technologies, about 14,000 base pairs can be acquired in 14 hours in an electrophoresis gel. The ultimate goal of 3 billion base pairs therefore poses a technological challenge and presents a need for high performance sequencing instruments. To this end, mass spectrometry can be used as a sequencing technique.

An important field emerging from genomics is proteomics. Proteomics concerns the study of all the proteins encoded for by genes. Like genomics, proteomics involves extremely complex mixtures of large biopolymers (proteins in this case) that need to be separated and identified. Current technologies mainly make use of 2-D electophoresis gels, which separate proteins based on both size and the isoelectric point of the proteins. These gels are labor intensive to prepare and time-consuming to run and analyze. Mass spectrometry offers a high-speed, high-sensitivity, low-labor alternative to separate, sequence, and identify complex mixtures of proteins.

Mass spectrometry allows the acquisition of molecular weights (measured in daltons) for every mass to charge (m/z) peak acquired, whereby the m/z ratio

is an intrinsic and condition-independent property of an ion. By eliminating the preparation of gels required with electrophoretic mobility analysis, mass spectrometry has the potential for requiring only milliseconds per analysis. By its nature, it is an intrinsically fast and accurate means for accurately accessing molecular weights.

Mass spectrometry requires that the analyte of interest be produced in the form of a gas phase ion, within the vacuum of a mass spectrometer for analysis. While achieving this is straightforward for small molecules using classical techniques (such as sublimation or thermal desorption) used in conjunction with an ionization method (such as electron impact), it is much less straight-forward for large biopolymers with essentially nonexistent vapor pressures. For this reason, the field of large-molecule mass spectrometry was extremely limited for many years. This situation changed dramatically with the discovery of two important new techniques for producing ions of large biomolecules (macromolecules), namely Matrix Assisted Laser Desorption-Ionization (MALDI) and Electrospray Ionization (ESI), whereby rapidly determining the mass of large molecules became feasible.

In MALDI mass spectrometry, a few hundred femtomoles of analyte are mixed on a probe tip with a small, organic, ultra-violet (UV) absorbing compound, the matrix. The analyte-matrix is dried to produce a heterogenous crystalline dispersion, and then irradiated with a brief (i.e., 10 ns) pulse of UV laser radiation in order to volatilize the sample and produce gas phase ions of the analyte amenable to mass spectrometric analysis. Because the UV pulse is at a wavelength that is absorbed by the matrix and not the analyte, the matrix is

vaporized, and analyte molecules become entrained in the resultant gas phase plume where they are ionized in gas phase proton transfer reactions. However, analyte fragmentation and poorly understood matrix effects occur during the MALDI process, thereby reducing molecular ion intensity and complicating the analysis and interpretation of the mass spectra. As a result, the mass range of this technique is limited; it frequently does not allow sequencing fragments longer then 35-100 base pairs in length.

Electrospray ionization mass spectrometry (ESI-MS), on the other hand, allows analysis of DNA without fragmentation. ESI-MS is characterized by an extremely gentle analyte desorption/ionization process that can leave even noncovalent bonds intact. This soft ionization allows analysis of intact DNA molecular ions. However, ESI-MS typically produces multiply charged ions, and as the number of possible charge states increases with the size of the analyte, this technique yields complex spectra for large molecules. For example, while ESI analysis of simple molecules may be accomplished using computer algorithms that transform the multiply charged mass spectra to "zero-charge" spectra, permitting easy visual interpretation thereof, as spectral complexity and chemical noise levels increase, these algorithms produce artificial peaks and miss analyte peaks with low signal intensity. Furthermore, each analyte yields a specific peak distribution and mixture spectra are therefore characterized by complex overlapping distributions for which the resultant spectra cannot be resolved without expensive high resolution mass spectrometers. This multiple charging and peak multiplicity in ESI-MS considerably limit the utility of this technique in the analysis of mixtures such as DNA sequencing ladders or

complex protein mixtures, and serious efforts to utilize ESI-MS as a sequencing tool have thus been hampered by the complexity of the resultant mass spectra.

To make ESI-MS more effective, it is desirable to decrease the charge of electrospray generated ions. Previous approaches to charge reduction in ESI have fallen into two major categories: modification of the solution conditions (i.e., buffer, pH, salts) and utilization of gas-phase reactions within an ion trap spectrometer. Altering solution conditions does not allow predictable and controllable manipulation of the charge state for all species present in a given mixture. With conventional ion trap techniques, the cation or anion used to reduce charge has to be "trapped" along with the analyte(s). This has the practical consequence of limiting the charge reduction to a narrow m/z range of ions. Thus, previous ion trap apparatuses are limited by the nature of the ion trap to a defined m/z range and are thus not amenable to the charge reduction of extremely large m/z ions. This is of course critical for reducing the charge of large DNA molecules.

As is evident from the foregoing, a need exists for a method of combining the simplicity of singly charged species spectra with the softness of ESI to efficiently and effectively allow high resolution mass spectral analysis of a mixture of a sample analyte solution containing a macromolecule of interest in a solvent wherein the method used is not limited to a low m/z range and wherein off-line sample purification or pre-separation are not required.

BRIEF SUMMARY OF THE INVENTION

The method of the present invention enables mass spectral analysis of a solution containing a macromolecule of interest by preparing a sample analyte

solution containing the macromolecule in a solvent, discharging, with the assistance of a nebulizing gas, the analyte solution through an orifice held at a high voltage in order to produce a plurality of analyte droplets that are multiply charged, evaporating the solvent in the presence of a bath gas in order to provide a plurality of macromolecule particles having multiple charges, exposing the bath gas proximal to the macromolecule particles to a radioactive alpha-particle emitting source that ionizes elements of the bath gas into bipolar ions, controlling the dwell time of the macromolecule particles in the bipolar ion mixture with the bath gas ions in order to reduce the multiply charged macromolecule particles to predominantly singly charged and no-charge neutral particles, and then analyzing the stream of singly charged macromolecule particles in a mass spectrometer.

More specifically, a sample analyte solution is placed into a vessel in an ESI source and discharged as an aerosol through an orifice held at a high potential. Due to a voltage differential between the spray tip orifice and the internal walls of the ESI source, an electrostatic field is created whereby charges accumulate at the surface of the emerging droplets. Charge reduction is achieved by exposure of the aerosol to a high concentration of bipolar ions (i.e., both positively and negatively charged ions) present in the neutralization chamber. Collisions between the charged aerosol and the bipolar ions in the bath gas result in the neutralization of the multiply charged electrospray ions. The rate of this process is controlled by varying the concentration of the bipolar ions in the bath gas and the degree of aerosol exposure to an ionization source such as Polonium (^{210}Po), a radioactive metallic element that emits alpha

particles to form an isotope of lead. This provides, in effect, the ability to "tune" the charge state of the electrospray generated ions. A practical consequence is the ability to control the charge distribution of electrospray generated ions such that the ions can be manipulated to consist principally of singly charged ions and neutrals, thereby simplifying mass spectral analysis of DNA and protein mixtures.

By the disclosed method, the present inventors have succeeded in using an ESI-TOF (Electrospray Ionization-Time of Flight) mass spectrometer to analyze particles ranging from 4 to 8 kDa in size In this technique, the particles in the continuous liquid flow from the electrospray source are desorbed and ionized. The resultant multiply charged species are then neutralized by passage through the radioactive neutralizing chamber whereby singly charged macromolecules result. As a result, the charge state of the ions generated in the electrospray chamber are reduced in a controlled manner whereby the stream of singly charged macromolecules is analyzed in a mass spectrometer such as an orthogonal time-of-flight (TOF) mass spectrometer, yielding high resolution mass spectra.

The method described herein decouples the ion production process from the neutralization process. This is important because it provides flexibility with respect to the electrospray conditions, which is critical to obtaining high-quality results, and it permits control over the degree of charge neutralization. In addition, with the approach presented here, the cation or anion used to reduce charge does not have to be "trapped" with the electrospray ions. This has the practical consequence of permitting the charge reduction to be performed on virtually any m/z ranges of ions, independent of the neutralizing cation or anion's

m/z value. In addition, because a specific anionic or cationic species is not required in the method of this invention, switching between positive and negative modes of electrospray is straightforward. This allows protein cations to be neutralized in positive ion mode or DNA anions to be neutralized in negative ion mode without having to change any instrumental conditions other than operating polarity.

It is thus one object of this invention to allow rapid analysis of mixtures of synthetic or biopolymers with high m/z ranges for a wide range of applications. It is another object of the present invention to accomplish the above objective without requiring a major change in standard operational procedures. It is yet another objective of the present invention to accomplish the above objectives with a minimal cost adjustment over traditional ESI, thereby permitting accurate, high speed, high resolution, and low cost effective mass determinations of DNA macromolecules without requiring preparation of a mixture on a column or being subject to the limitations of traditional ion traps.

The foregoing and other objects, advantages, and aspects of the present invention will become apparent from the following description. In the description, reference is made to the accompanying drawings which form a part hereof, and in which there is shown, by way of illustration, a preferred embodiment of the present invention. Such embodiment does not necessarily represent the full scope of the invention, however, and reference must also be made to the claims herein for properly interpreting the scope of this invention.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Fig. 1 is a block diagram of the apparatus used in the method of this invention;

Fig. 2 is an expanded cross-sectional partial view of the apparatus used in the method of this invention;

Fig. 3 is an exploded cross-sectional view of the spray tip of the capillary of the ESI source;

Fig. 4 is a front view of the spray tip of the capillary of the ESI source;

Fig. 5 is a simplified cross-sectional view of a ESI-TOF MS used in the method of electrospray analysis of the present invention;

Fig. 6 depicts the effect of charge state reduction on ubiquitin as a function of exposed area of the alpha particle source, whereby Fig. 6-A shows mass spectra with the alpha particle 0% exposed, Fig. 6-B shows mass spectra with the alpha particle 17.5% exposed, and Fig. 6-C shows mass spectra with the alpha particle 100% exposed;

Fig. 7 depicts the effect of charge state reduction on a mixture of insulin, ubiquitin, and cytochrome c, whereby Fig. 7-A shows mass spectra without charge reduction and Fig. 7-B shows mass spectra with charge reduction; and

Fig. 8 depicts the effect of charge state reduction on a mixture of three oligonucleotides, a 15 mer d(TGTAAAACGACGGCC), a 21 mer d(TGTAAAAC GACGGCCAGTGCC), and a 27 mer d(TGTAAAACGACGGCCAGTGCCAAGC TT), whereby Fig. 8-A shows mass spectra without charge reduction and Fig. 8-B shows mass spectra with charge reduction.

DETAILED DESCRIPTION OF THE INVENTION

An apparatus used in the method of the present invention comprises three primary components, depicted generally by the block diagrams of Fig. 1, wherein a positive-pressure ESI source 100 is operably linked to a charge reduction source 200, which is, in turn, operably linked to a TOF mass spectrometer 300.

Referring now to the ESI source 100 shown in Fig. 2, a protective casing 102 houses a 0.5 mL polypropylene vessel 104 within which a sample analyte 106 is placed. In the preferred embodiment, the ESI source 100 comprises a 24 cm fused-silica polyamide coated capillary 108 (150 mm o.d., 25 mm i.d.) having an inlet 110 at one end and a spray tip 112 at the other end.

As shown in Fig. 3, the spray tip 112 of the capillary 108 is conically ground to a cone angle 114 (angle between the capillary axis 116 and the cone surface 118) of approximately 25-35 degrees in order to form a nebulizer. Although many types of nebulizers are known, including ultrasonic, pneumatic, frit, and thermospray, an electrospray nebulizer is preferred because of its ability to generate small and uniform electrically charged droplets at its spray tip 112. Accordingly, Fig. 4 shows a front view of a spray tip 112 of an electrospray nebulizer, as taken along line 4-4 in Fig. 3.

Referring again to Fig. 2, the inlet 110 of the capillary 108 is immersed in a solution containing the sample analyte 106 whereby a pressurized gas cylinder applies a positive pressure of 7 psi (49 kpa) to the sample analyte 106 to produce typical flow rates of 0.13 µL/min through the capillary 108 into near-atmospheric pressure inside the charge reduction source 200. The analyte 106 is maintained at a high potential such as 4500 V (positive for positive ion mode,

negative for negative ion mode) by means of a platinum electrode 120 immersed therein.

In a preferred embodiment, the charge reduction source 200 is cylindrical, preferably with diameter of 1.9 cm and a length of 4.3 cm. The charge reduction source 200 comprises an upstream spray chamber 202 and an adjacent downstream charge neutralization chamber 204, wherebetween an electrically conductive, Teflon coated plate or wall 203 separates the chambers such that the plate or wall 203 can be biased to attract newly formed charged droplets emerging from the spray tip 112 towards the neutralization chamber 204.

The opposite end of the spray chamber 202 comprises a spray manifold 206 through which a plurality of orifices traverse. The capillary 108 of the ESI source 100 passes through one orifice and is held in place by support members 208. As the analyte 106 is sprayed out of the spray tip 112, it is stabilized against corona discharge by a sheath/nebulizer gas of CO_2, which typically flows between 0.1 - 2 L/min through a stainless steel sheath gas inlet tube (1.5 mm i.d.) 210 that is concentric with the silica capillary 108. Typically, the sheath gas is monitored and controlled by a flow meter 212 and a filter 214 before delivery through the sheath gas inlet tube 210 and into the spray chamber 202.

The other orifices of the spray manifold 206 allow passage of a bath gas such as nitrogen or carbon dioxide or medical air via a plurality of bath gas inlet tubes 216 through which the bath gas typically flows after passage through a flow meter 218 and filter 220. Typical flow rates are often 1-4 L/min.

In the ESI-MS technique, electrospray ionization occurs by spraying the analyte 106 at a controlled rate out of the spray tip 112, which is maintained at a

high electric potential. Typical flow rates are of the order of 0.1 - 1 μL/min. Via a voltage differential between the spray tip 112 and the internal walls 222 of the spray chamber 202, an electrostatic field is created whereby charges accumulate at the surface of the droplets emerging from the spray tip 112. Because solvent evaporates from each droplet as the droplets travel towards the neutralization chamber 204, they shrink, and the charge density on each droplet surface increases until the Rayleigh limit is reached, at which point electrostatic Coulomb repulsion forces between the charges approach in magnitude the droplet's cohesive forces such as surface tension. The resulting instability causes a "Coulomb explosion" whereby the original droplet, sometimes referred to as the parent or primary droplet, disintegrates into smaller droplets, sometimes referred to as daughter droplets. As the parent droplet disintegrates into daughter droplets, a substantial proportion of the total charge is removed. And as the daughter droplets shrink further in the drying gas, they too quickly reach the Rayleigh limit and undergo their own Coulomb explosion to give way to even smaller droplets. It is believed that the droplets successively disintegrate following this cascade mechanism until the analyte 106 molecules contained in the droplet are entirely desorbed in the gas phase.

Flow of the CO_2 sheath gas through the sheath gas inlet tube 210 is controlled by the flow meter 212 to shield against coma discharge at the spray tip, and flow of the bath gas through the bath gas inlet tubes 216 is controlled by the flow meter 218 both to control the rate of movement of the droplets through the spray chamber 202 and to dry the droplets.

Within the neutralization chamber 204, a 3.1 cm diameter hole is cut into the casing of the cylinder into which a Polonium or Polonium-like alpha emitting source 226 is attached. The alpha particles produced by radio isotopic sources such as ^{210}Po and ^{241}Am react with components of the sheath and bath gases, producing a variety of both positively and negatively charged ions (i.e., bipolar ions). The bipolar ions react with and neutralize other ionic species, such as the multiply charged analyte molecules from the ES ionization, whereby sufficient ionization is produced to reach steady-state distribution inside the neutralization chamber 204.

Hence, multiply charged analyte ions from the spray tip 112 entering the neutralization chamber 204 rapidly lose their charge, yielding mostly neutral and singly charged species. Because the droplets remain uniform in size, a monodisperse aerosol will be presented to the differential mobility analyzer 300 or other instrument.

Two factors are important in determining the degree of charge neutralization occurring within the neutralizing chamber 204: the alpha particle flux from the radio-active source 226 and the dwell time of the aerosol particles in the neutralization chamber 204. The alpha particle flux is controlled by an alpha source attenuator 224 that can shield the alpha source 226 from the neutralization chamber 204. For example, in a preferred embodiment, the alpha particle flux is modulated by placing a plurality of thin (i.e., typically 0.005 inches thick) brass disks with various numbers of holes of known areas drilled therein between the ^{210}Po source 226 and the neutralization chamber 204, whereby the alpha source 226 is completely shielded by a brass disk with no holes, and is

shielded proportionally to the exposed surface area when holes are present in the disks.

As previously discussed, the dwell time of the aerosol particles can be controlled by varying the flow rate of the bath gas through the bath gas inlet tubes 216. For example, by varying the flow rate of the bath gas, a lower flow rate of bath gas leads to longer dwell time and more extensive neutralization and a higher flow rate of bath gas leads to shorter dwell time and less extensive neutralization. By balancing the dwell time with the alpha particle source exposure, a charge distribution of a "neutral" aerosol is obtained, whereby the bath gas ions and alpha particles reduced the multiply charged macromolecule particles to predominantly singly and no-charge macromolecule particles. This balance will permit analysis of mixture spectra.

Referring now to the preferred embodiment in Fig. 5, the neutralized aerosol exits the neutralization chamber 204 through a 3 mm diameter outlet 230. A portion of this aerosol enters the mass spectrometer through the MS atmospheric pressure to vacuum interface for subsequent analysis.

The approach described herein is readily implemented by simple modification to the ESI source, and it is thus adaptable to virtually any mass analyzer. However, the high mass of common proteins and nucleic acids can quickly exceed the m/z ranges accessible with most mass analyzer instruments, and for this reason, an orthogonal TOF system is preferred because of the high intrinsic m/z range of this type of analyzer. For example, the reduction of charge state described above necessarily increases the m/z ratio of the ions being analyzed. In conventional ESI-MS, even very large molecules (i.e., megadaltons

in size) are produced with m/z ratios below 4,000, enabling analysis thereof with a variety of mass analyzers. However, with mixture charge reduction, the relatively high mass of common proteins and nucleic acids can quickly exceed the m/z range accessible with most instrument configurations. An orthogonal time-of-flight mass spectrometer, on the other hand, is characterized by the very high intrinsic m/z range of TOF analysis. For instance, the mass spectrometer 300 in a preferred embodiment is the commercially available PerSeptive Biosystems Mariner Workstation, an orthogonal TOF mass spectrometer with a m/z range of 25,000 amu and a measured external mass accuracy of better than 10 ppm.

In the preferred embodiment, the chosen analyzer 300 is interfaced to the charge reduction source 200 through a plurality of skimmer orifices, allowing the transport of the aerosol from atmospheric pressure into the high vacuum region of the spectrometer 302. The skimmer orifices 302 are further connected to a plurality of focusing and pulsing elements. A quadrupole focusing lens 304 is used to initially focus the ions. The focused ion packets are accelerated down an electric field free region 314 via a series of ion optic elements and pulsing electronics 306, 308, 310, and 312.

All ions receive the same kinetic energy as a result of this process. The kinetic energy is proportional to the product of the mass and velocity of the ion, thus heavier ions will travel slower then lighter ions. Hence, the arrival times of the ions at the end of the flight tube are separated in time proportional to their mass. The arrival of the ions is typically detected with a microchannel-based detector, the output signal of which can be measured as a function of time by a

1.3 Ghz time-to-digital converter 320. The appropriate time measurements are transmitted for storage into and analysis by a computer 322.

Using a calibrant, the computer 322 can derive the mass of the arriving ions by converting flight times to molecular weights. By techniques known in the art, the computer can be programmed to run software that outputs the mass spectra as smoothed by convolution with a Gaussian function. Resultant mass spectra are depicted in the graphs of Figs. 6-8, whereby mass (measured in units corresponding to m/z) is depicted on the x-axis and intensity (measured in arbitrary units) is depicted on the y-axis.

With reference now to Fig. 6, a series of positive ion mass spectra was obtained in the analysis of the protein ubiquitin (8564.8 Amu; 5µM in 1:1 H_20:acetonitrile, 1% acetic acid) at increasing levels of exposure to the ^{210}Po particle source 226. The averaged mass spectra shown were obtained over a 250 second time period at a spectral acquisition rate of 10kHZ, consuming 0.54 µL (2.7 pmol) of sample.

As shown in Fig. 6-A, with the ^{210}Po source 226 completely shielded, a typical ESI charge distribution is observed, with six major charge states evident (+7 to +2) and with the peak of the distribution corresponding to the +5 charge state. As shown in Fig. 6-B, where the degree of exposure to the ^{210}Po source 226 was increased to 17.5% by using a different alpha source attenuator 224, the charge state distribution moved toward lower and fewer charge states, until, as shown in Fig. 6-C, with the ^{210}Po source 226 completely unshielded, only two major charge states were observed, with the major peak corresponding to the +1 charge state. This result demonstrates the feasibility of obtaining high resolution

TOF mass spectra by controlling the charge state by way of varying macromolecule exposure to radioactive ionizing sources 226 such as Polonium.

The effect of charge reduction on the analysis of a simple protein mixture by time-of-flight ESI-MS is shown in Fig. 7. An equimolar mixture of three proteins (insulin, 5733.5 amu; ubiquitin, 8564.8 amu; and cytochrome c, 12360 amu) was prepared and mass analyzed with and without charge reduction. The mass spectra shown were obtained over a 250 second time period at a spectral acquisition rate of 10kHz, consuming 0.54 µL (2.7 pmol) of sample.

The result obtained in the absence of charge reduction is shown in Fig. 7-A, which corresponds to a fairly typical ESI mass spectrum for such a mixture. The mass spectrum is complex, containing about 50 peaks, 18 of which correspond to the various charge states of the proteins as shown in the figure. In contrast, the spectrum shown in Fig. 7-B exhibits only eight major peaks, which are readily assigned by those skilled in the art. This result demonstrates the heretofore unknown reduction of spectral complexity in mixture analysis afforded by charge reduction. In Fig. 7-B, the absence of the acetate adduct on the +2 charge state of cytochrome c can be attributed to collision activated dissociation (CAD) in the region proximal to the skimmer orifices 302.

Finally, the effect of charge reduction on the analysis of a simple oligonucleotide mixture by the method of this invention is shown in Fig. 8. An equimolar mixture of three oligonucleotides 15, 21, and 27 nucleotides in length was prepared and mass analyzed with and without charge reduction. Each oligonucleotide was at a concentration of 10 µM in 3:1 $H_20:CH_3OH$, 400 mM 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), adjusted to pH 7 with triethylamine.

The HFIP buffer was found to yield the least Na^+ and K^+ oligonucleotide adduction of any buffer tested and was used for that reason. The averaged mass spectra shown were obtained over a 500 second time period at a spectral acquisition rate of 10kHz, consuming 1.08 µL (5.4 pmol) of sample.

The result obtained in the absence of charge reduction (i.e., with the ^{210}Po source 226 fully shielded) is shown in Fig. 8-A. Without charge reduction, the ESI mass spectrum obtained for such a mixture yields a complex spectra, with overlapping peaks corresponding to several different charge states for the three oligonucleotides in the mixture. Many other peaks due to fragmentation are also observed. Analysis of the spectra of such a mixture is compromised by the variety of charge states present in the sample, yielding too many overlapping spectrum peaks to permit effective discrimination amongst the various species present. The effect of charge reduction, on the other hand, is shown in Fig. 8-B, in which charge reduction greatly simplifies the mass spectrum, with only six major peaks evident, corresponding to the singly and doubly charged ions for each oligonucleotide.

All of the unreduced charge spectra (Figs. 6-A, 7-A, and 8-A) show a number of peaks in the low m/z region that do not correspond to charge states of the analytes, but that disappear in the charge-reduced spectra (Figs. 6-B, 7-B, and 8-B). The m/z ratios and isotopic distributions of these peaks correspond predominantly to singly charged fragment ions, with only a few multiply charged fragment ions (assignments not shown). The disappearance of these peaks with charge reduction is advantageous in a practical sense because it constitutes a substantial reduction in the "chemical noise" of the system.

Because the charge reduction process converts ions to neutral species that are not detected by the analyzer 300, the signal intensities in the charge-reduced spectra are substantially lower than those in the non charge-reduced spectra. Conversely, however, the reduction in chemical noise described above and simplification of the spectra both tend to increase detection sensitivity.

The spirit of the present invention is not limited to any embodiment described above. Rather, the details and features of an exemplary embodiment were disclosed as required. Without departing from the scope of this invention, other modifications will therefore be apparent to those skilled in the art. Thus, it must be understood that the detailed description of the invention and drawings were intended as illustrative only, and not by way of limitation.

To apprise the public of the scope of this invention, the following claims are made:

CLAIMS

What is claimed is:

1. A method of preparing mass spectra of macromolecules comprising the steps of:

(a) preparing a sample analyte solution containing a macromolecule of interest in a solvent;

(b) discharging the analyte solution through an orifice held at a high voltage to produce a plurality of analyte droplets having multiple charges;

(c) evaporating the solvent in the presence of a bath gas to provide a plurality of macromolecule particles having multiple charges;

(d) exposing the bath gas about the macromolecule particles to a radioactive source emitting particles that ionize elements of the bath gas into bipolar ions;

(e) controlling the dwell time of the macromolecule particles within the bipolar ions with the bath gas to reduce the multiply charged macromolecule particles to predominantly singly charged and no-charge neutral macromolecule particles; and

(f) analyzing the stream of singly charged macromolecules in a mass spectrometer.

2. The method of claim 1 wherein the bath gas ions include both positive and negative ions.

3. The method of claim 1 wherein the radioactive source produces alpha particles.

4. The method of claim 3 wherein the radioactive source is ^{210}Po.

5. The method of claim 1 wherein the bath gas is selected from the group consisting of: nitrogen, carbon dioxide, or medical air.

6. The method of claim 1 wherein step (d) controls the flux of particles from the radioactive source with a blocking disk having apertures of known area.

7. The method of claim 1 wherein step (d) occurs in a chamber held at the same voltage as the orifice.

8. The method of claim 1 wherein step (f) employs an orthogonal time of flight mass spectrometer.

9. A method of reducing fragmentation of long chain macromolecules in electrospray mass spectrometry comprising the steps of:

(a) preparing a sample analyte solution containing a macromolecule of interest in a solvent;

(b) discharging the analyte solution through an orifice held at a high voltage to produce a plurality of analyte droplets having multiple charges;

(c) evaporating the solvent in the presence of a bath gas to provide a plurality of macromolecule particles having multiple charges;

(d) exposing the bath gas about the macromolecule particles to a radioactive source emitting particles that ionize elements of the bath gas into bipolar ions;

(e) controlling the dwell time of the macromolecule particles within the bipolar ions with the bath gas to reduce the multiply charged macromolecule

particles to predominantly singly changed and no-charge neutral macromolecule particles; and

(f) analyzing the stream of singly charged macromolecules in a mass spectrometer.

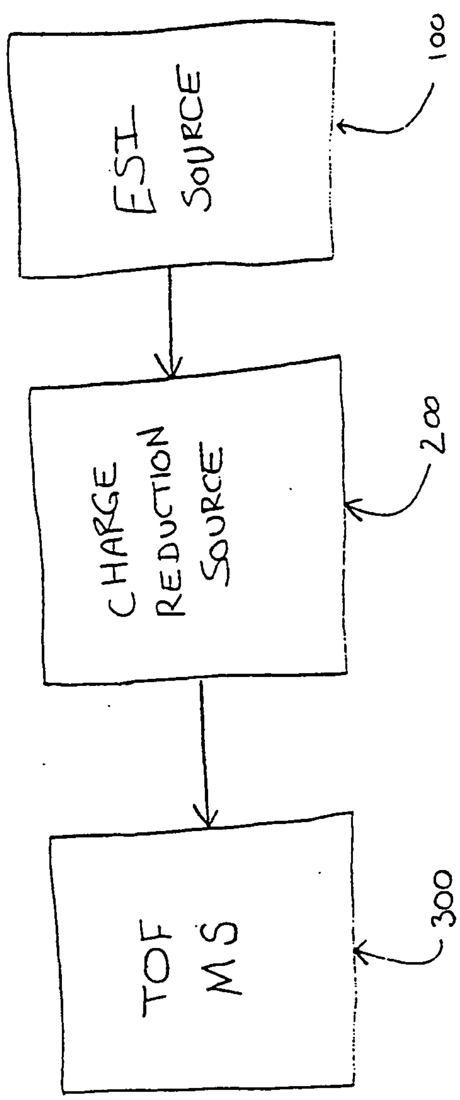


FIG. 1

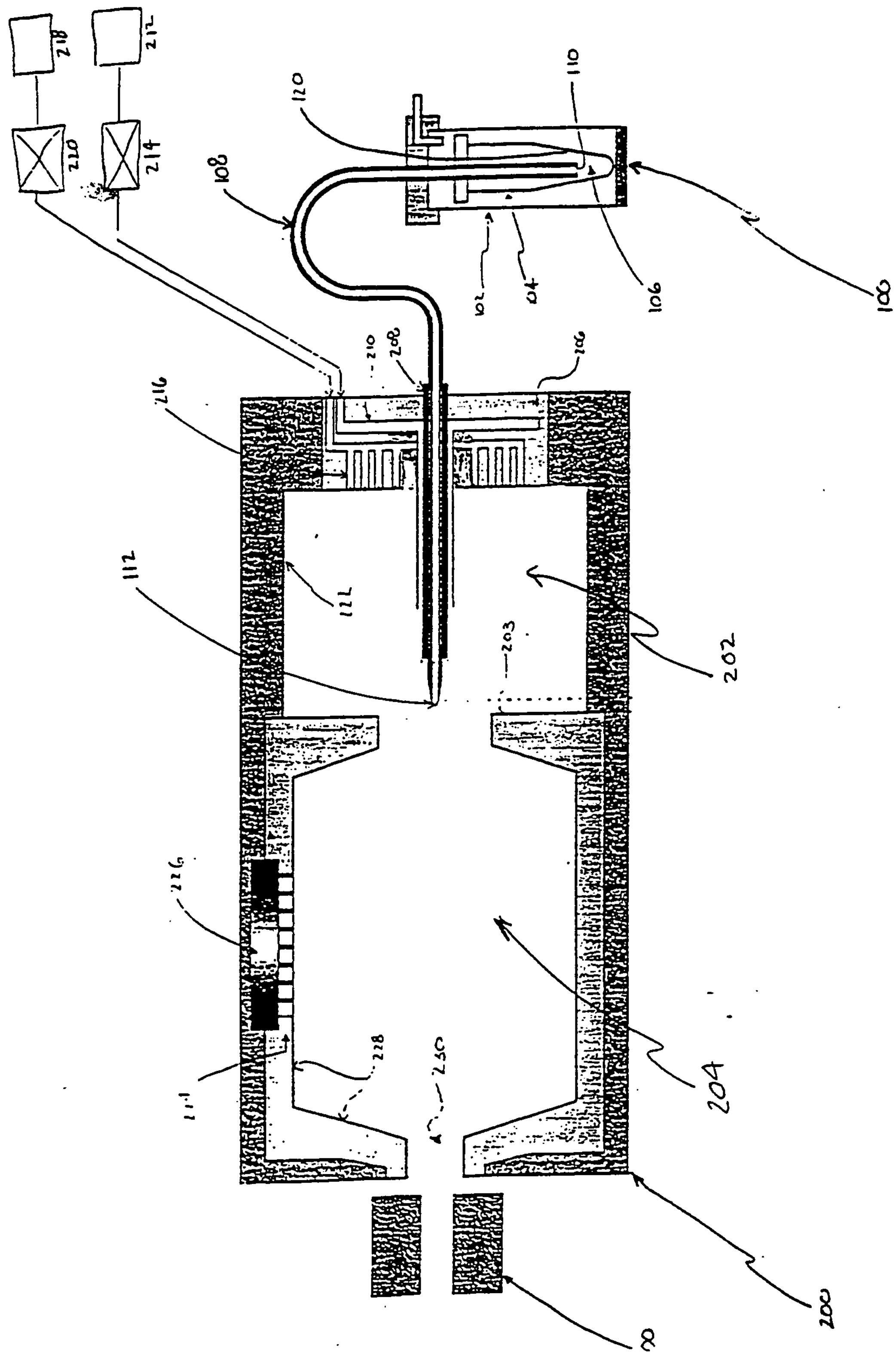

100
102
104
106
108
110
120
112
122
200
202
203
204
208
210
212
214
216
218
226
228
230

FIG. 2

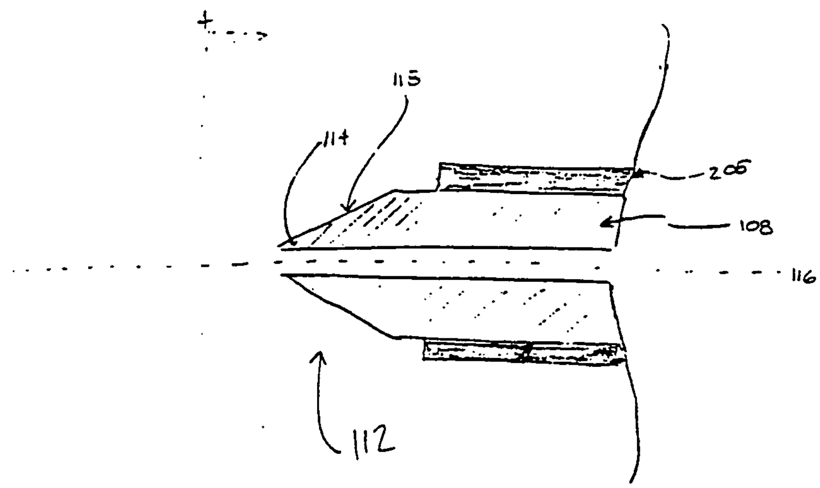


FIG. 3

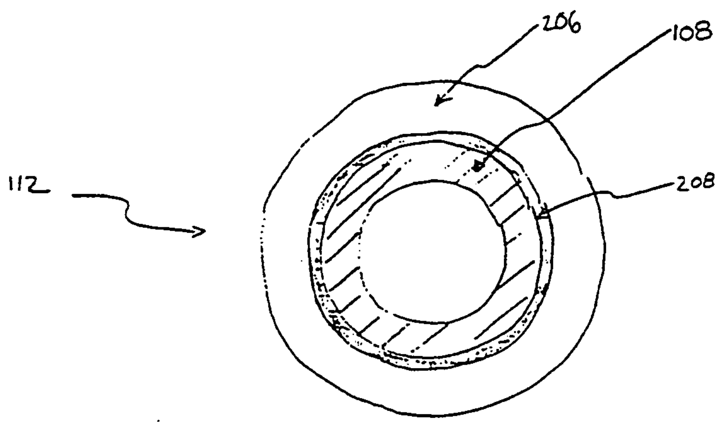


FIG 4

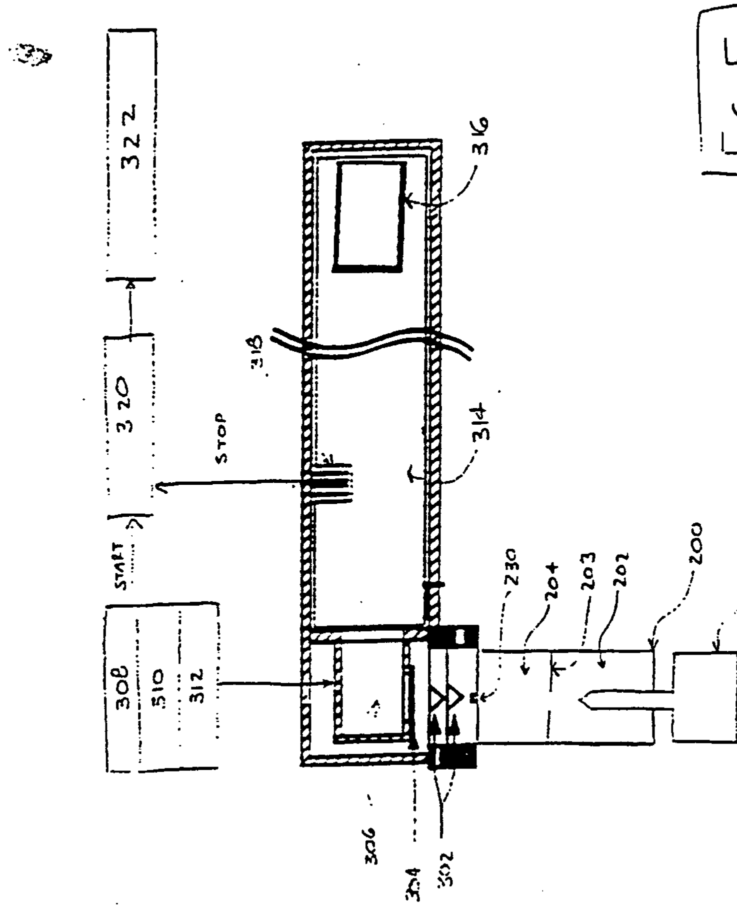
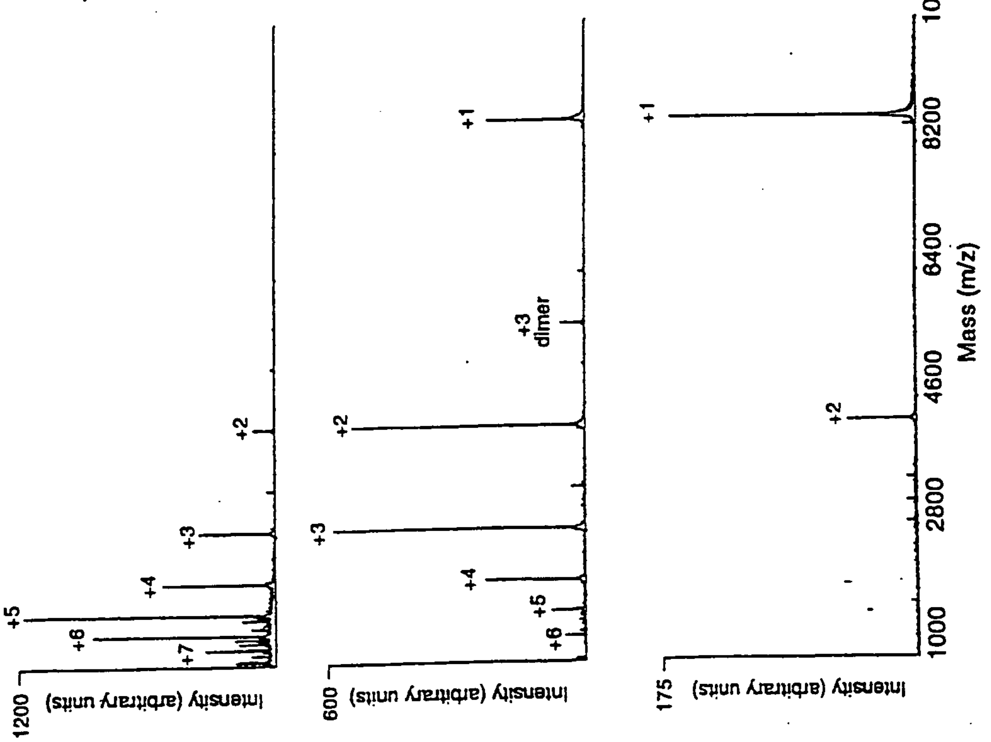

322
320
STOP
START
308
310
312
316
318
314
230
204
203
202
200
100
306
304
302

FIG. 5

FIG. 6-A

FIG. 6-B

FIG. 6-C



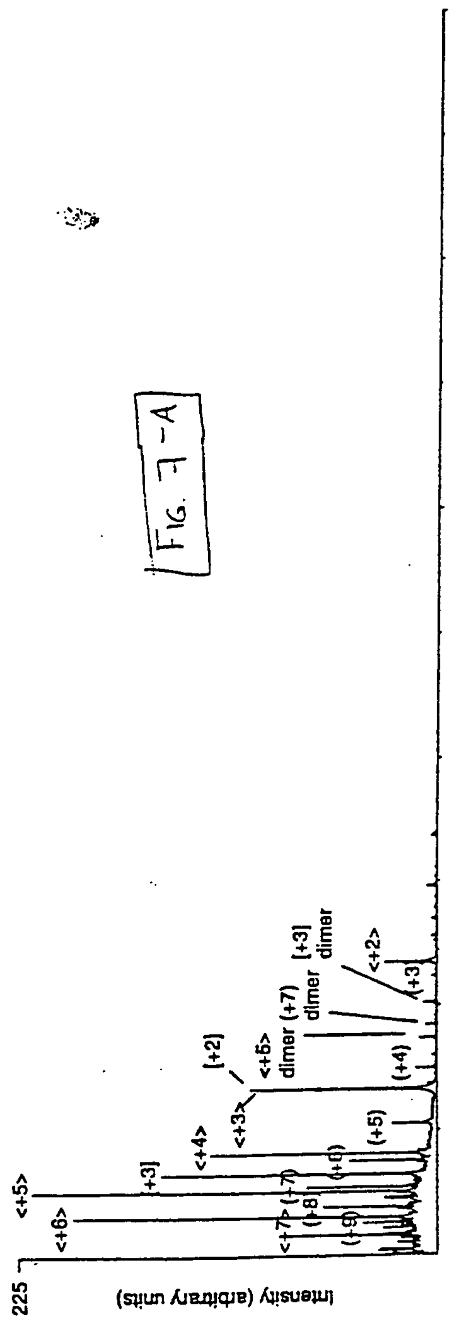

225
Intensity (arbitrary units)
<+5>
<+6>
[+3]
<+4>
<+3>
[+2]
<+5>
dimer
(+7)
dimer
[+3]
dimer
<+2>
(+4)
(+5)

FIG. 7-A

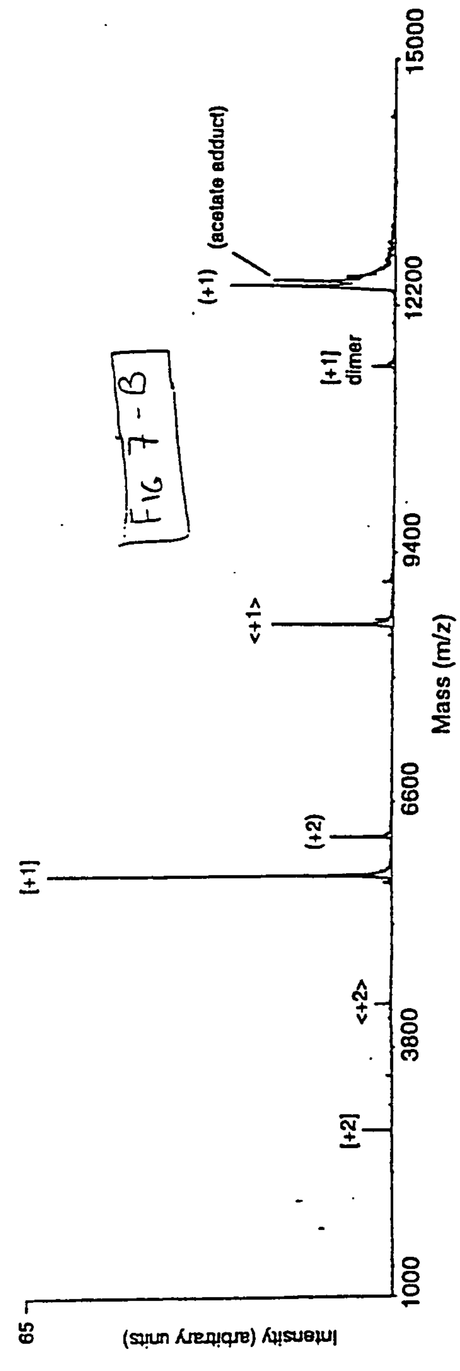

65
Intensity (arbitrary units)
[+2]
<+2>
[+1]
(+2)
<+1>
[+1]
dimer
(+1)
(acetate adduct)
1000
3800
6600
9400
12200
15000
Mass (m/z)

FIG. 7-B

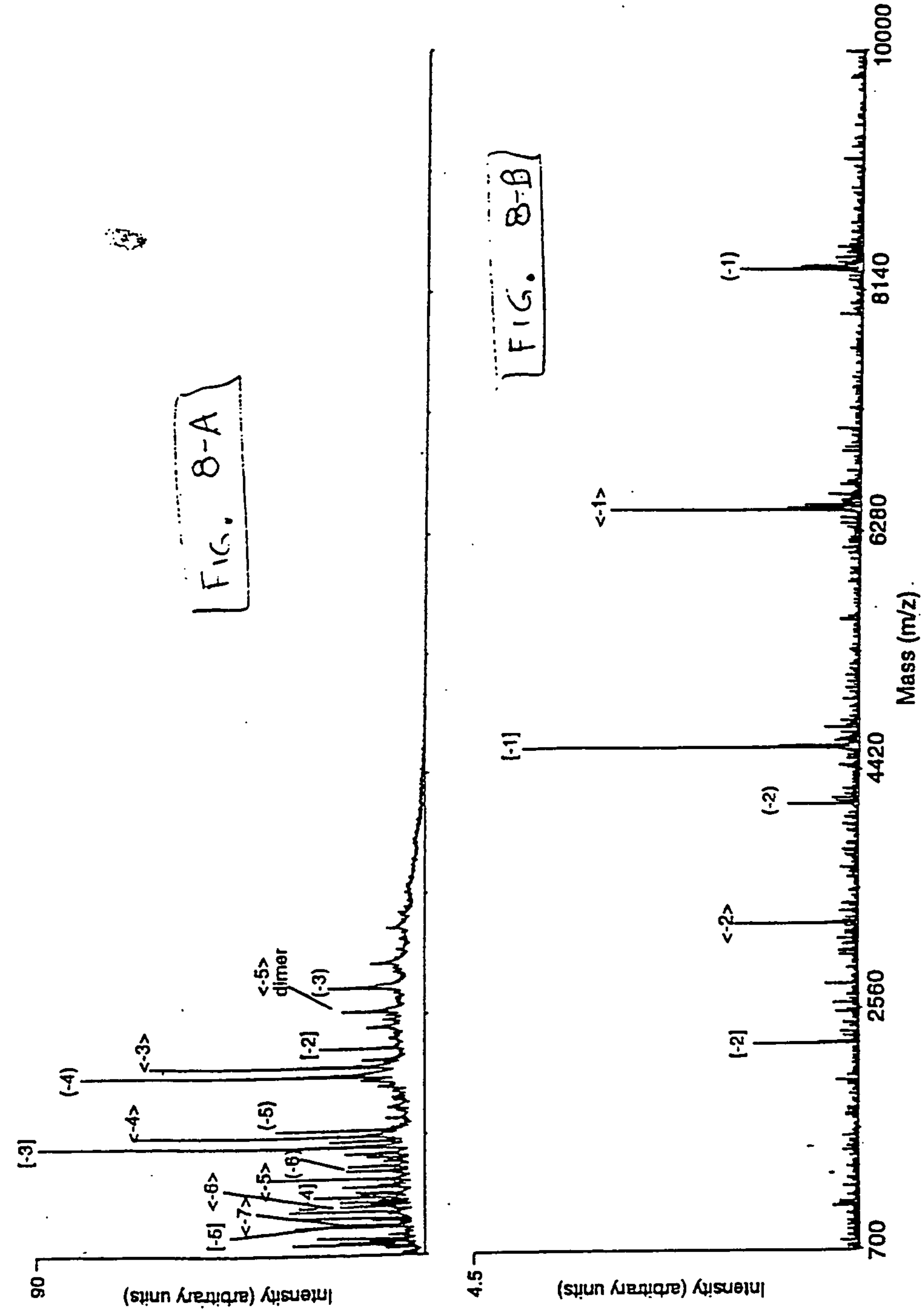

90
Intensity (arbitrary units)
[-3]
(-4)
<-4>
<-3>
[-5]
<-7>
<-6>
<-5>
[-4]
(-6)
(-5)
[-2]
<-5>
dimer
(-3)
4.5
Intensity (arbitrary units)
[-2]
<-2>
(-2)
[-1]
<-1>
(-1)
700
2560
4420
6280
8140
10000
Mass (m/z)

FIG. 8-A

FIG. 8-B